

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07K 14/47, C12N 15/12, 15/11, C12Q 1/68, C07K 14/435	A1	(11) International Publication Number: WO 00/47617 (43) International Publication Date: 17 August 2000 (17.08.00)
(21) International Application Number: PCT/US00/03425 (22) International Filing Date: 9 February 2000 (09.02.00) (30) Priority Data: 60/119,228 9 February 1999 (09.02.99) US 60/158,458 8 October 1999 (08.10.99) US (71) Applicant: LEXICON GENETICS INCORPORATED [US/US]; 400 Research Forest Drive, The Woodlands, TX 77381 (US). (72) Inventors: TURNER, C., Alexander, Jr.; 67 Winter Wheat Place, The Woodlands, TX 77381 (US). MATHUR, Brian; 12000 Sawmill Road #2014, The Woodlands, TX 77380 (US). ZAMBROWICZ, Brian; 18 Firethorne Place, The Woodlands, TX 77382 (US). SANDS, Arthur, T.; 163 Bristol Bend Circle, The Woodlands, TX 77382 (US). (74) Agent: ISHIMOTO, Lance; Intellectual Property, Lexicon Genetics Incorporated, 4000 Research Forest Drive, The Woodlands, TX 77381 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: HUMAN UNCOUPLING PROTEINS AND POLYNUCLEOTIDES ENCODING THE SAME (57) Abstract Novel human polynucleotide and polypeptide sequences are disclosed that can be used in therapeutic, diagnostic, and pharmacogenomic applications.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

HUMAN UNCOUPLING PROTEINS AND POLYNUCLEOTIDES ENCODING THE SAME

1. INTRODUCTION

5 The present invention relates to the discovery, identification, and characterization of novel human polynucleotide sequences and the novel polypeptides encoded thereby. The invention encompasses the described polynucleotides, host cell expression systems, the encoded
10 proteins or polypeptides, and fusion proteins and peptides derived therefrom, antibodies to the encoded proteins or peptides, and genetically engineered animals that do not produce a functional product of the disclosed sequences, or over express the disclosed sequences, as well as antagonists
15 and agonists of the described proteins, along with other compounds that modulate the expression or activity of the proteins encoded by the disclosed polynucleotides that can be used for diagnosis, drug screening, clinical trial monitoring, the treatment of physiological or behavioral disorders, or to
20 otherwise improve quality of life.

2. BACKGROUND OF THE INVENTION

Uncoupling proteins (UCPs) are found in the mitochondria, but are encoded within the nucleus. In the mitochondria, UCPs uncouple, or regulate, the gradient that drives energy
25 production in the cell/body. As such, UCPs effectively modulate the efficiency of energy production in the body, and hence body metabolism. Given the role of UCPs in the body, they are thought to be important targets for the study of thermogenesis, obesity, cachexia, and other metabolically
30 related physiological functions, diseases, and disorders.

3. SUMMARY OF THE INVENTION

The present invention relates to the discovery, identification, and characterization of nucleotides that encode novel human proteins, and the corresponding amino acid
35 sequences of these proteins. The novel human proteins (NHPs) described for the first time herein share structural

similarity with uncoupling proteins from a variety of animal species.

The novel human nucleic acid (cDNA) sequences described herein encode proteins/open reading frames (ORFs) of 291 and 5 293 amino acids in length (see respectively SEQ ID NOS: 2 and 4).

The invention also encompasses agonists and antagonists of the described NHPs including small molecules, large molecules, mutant NHPs, or portions thereof that compete with 10 native NHPs, NHP peptides, and antibodies, as well as nucleotide sequences that can be used to inhibit the expression of the described NHPs (e.g., antisense and ribozyme molecules, and gene or regulatory sequence replacement constructs) or to enhance the expression of the described NHPs 15 (e.g., expression constructs that place the described gene under the control of a strong promoter system), and transgenic animals that express a NHP transgene, or "knock-outs" (which can be conditional) that do not express a functional NHP. A gene trapped knockout ES cell line has been produced that 20 mutates a murine ortholog of the described sequences.

Further, the present invention also relates to processes for identifying compounds that modulate, i.e., act as agonists or antagonists, of NHP expression and/or NHP activity that utilize purified preparations of the described NHP and/or NHP 25 product, or cells expressing the same. Such compounds can be used as therapeutic agents for the treatment of any of a wide variety of symptoms associated with biological disorders or imbalances.

30 4. DESCRIPTION OF THE SEQUENCE LISTING AND FIGURES

The Sequence Listing provides the sequences of the NHP ORFs encoding the described NHP amino acid sequences.

5. DETAILED DESCRIPTION OF THE INVENTION

35 The cDNA sequences (SEQ ID NOS:1 and 3) and deduced amino acid sequences (SEQ ID NOS:2 and 4) of the described NHPs are presented in the Sequence Listing. The NHP cDNA sequences were obtained from human lymph node, kidney, and fetal brain

cDNA libraries (Edge Biosystems, Gaithersburg, MD) using probes and/or primers generated from gene trapped sequence tags and a human homolog of the described NHPs. RT-PCR analysis has indicated that expression of the described NHPs can be detected in, *inter alia*, human cerebellum, spinal cord, thymus, spleen, lymph node, bone marrow, trachea, lung, kidney, fetal liver, prostate, testis, thyroid, salivary gland, stomach, heart, uterus, and mammary gland, with particularly strong expression in kidney, adrenal gland, and skeletal muscle. The above expression studies were largely verified by Northern analysis which also detected particularly strong expression in human skeletal muscle, heart, adrenal gland, and kidney.

The present invention encompasses the nucleotides presented in the Sequence Listing, host cells expressing such nucleotides, the expression products of such nucleotides, and: (a) nucleotides that encode mammalian homologs of the described genes, including the specifically described NHPs, and the NHP products; (b) nucleotides that encode one or more portions of a NHP that correspond to functional domains of the NHP, and the polypeptide products specified by such nucleotide sequences, including but not limited to the novel regions of any active domain(s); (c) isolated nucleotides that encode mutant versions, engineered or naturally occurring, of a described NHP in which all or a part of at least one domain is deleted or altered, and the polypeptide products specified by such nucleotide sequences, including but not limited to soluble proteins and peptides in which all or a portion of the signal sequence is deleted; (d) nucleotides that encode chimeric fusion proteins containing all or a portion of a coding region of a NHP, or one of its domains (e.g., a receptor or ligand binding domain, accessory protein/self-association domain, etc.) fused to another peptide or polypeptide; or (e) therapeutic or diagnostic derivatives of the described polynucleotides such as oligonucleotides, antisense polynucleotides, ribozymes, dsRNA, or gene therapy constructs comprising a sequence first disclosed in the Sequence Listing. As discussed above, the present invention

includes: (a) the human DNA sequences presented in the Sequence Listing (and vectors comprising the same) and additionally contemplates any nucleotide sequence encoding a contiguous NHP open reading frame (ORF), or a contiguous exon
5 splice junction first described in the Sequence Listing, that hybridizes to a complement of a DNA sequence presented in the Sequence Listing under highly stringent conditions, e.g., hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in
10 0.1xSSC/0.1% SDS at 68°C (Ausubel F.M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3) and encodes a functionally equivalent gene product. Additionally contemplated are any nucleotide
15 sequences that hybridize to the complement of the DNA sequence that encode and express an amino acid sequence presented in the Sequence Listing under moderately stringent conditions, e.g., washing in 0.2xSSC/0.1% SDS at 42°C (Ausubel et al., 1989, *supra*), yet still encode a functionally equivalent NHP
20 product. Functional equivalents of a NHP include naturally occurring NHPs present in other species and mutant NHPs whether naturally occurring or engineered (by site directed mutagenesis, gene shuffling, directed evolution as described in, for example, U.S. Patent No. 5,837,458). The invention
25 also includes degenerate nucleic acid variants of the disclosed NHP polynucleotide sequences.

Additionally contemplated are polynucleotides encoding a NHP ORF, or its functional equivalent, encoded by a polynucleotide sequence that is about 99, 95, 90, or about 85
30 percent similar or identical to corresponding regions of the nucleotide sequences of the Sequence Listing (as measured by BLAST sequence comparison analysis using, for example, the GCG sequence analysis package using standard default settings).

The invention also includes nucleic acid molecules,
35 preferably DNA molecules, that hybridize to, and are therefore the complements of, the described NHP gene nucleotide sequences. Such hybridization conditions may be highly stringent or less highly stringent, as described above. In

instances where the nucleic acid molecules are deoxyoligonucleotides ("DNA oligos"), such molecules are generally about 16 to about 100 bases long, or about 20 to about 80, or about 34 to about 45 bases long, or any variation or combination of sizes represented therein that incorporate a contiguous region of sequence first disclosed in the Sequence Listing. Such oligonucleotides can be used in conjunction with the polymerase chain reaction (PCR) to screen libraries, isolate clones, and prepare cloning and sequencing templates, etc..

Alternatively, such NHP oligonucleotides can be used as hybridization probes for screening libraries, and assessing gene expression patterns (particularly using a micro array or high-throughput "chip" format). Additionally, a series of the described NHP oligonucleotide sequences, or the complements thereof, can be used to represent all or a portion of the described NHP sequences. The oligonucleotides, typically between about 16 to about 40 (or any whole number within the stated range) nucleotides in length may partially overlap each other and/or the NHP sequence may be represented using oligonucleotides that do not overlap. Accordingly, the described NHP polynucleotide sequences shall typically comprise at least about two or three distinct oligonucleotide sequences of at least about 18, and preferably about 25, nucleotides in length that are each first disclosed in the described Sequence Listing. Such oligonucleotide sequences may begin at any nucleotide present within a sequence in the Sequence Listing and proceed in either a sense (5'-to-3') orientation vis-a-vis the described sequence or in an antisense orientation.

For oligonucleotide probes, highly stringent conditions may refer, for example, to washing in 6xSSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos). These nucleic acid molecules may encode or act as NHP gene antisense molecules, useful, for example, in NHP gene regulation (for and/or as antisense primers in amplification reactions of NHP gene nucleic acid sequences). With respect to NHP gene regulation, such techniques can be used to

regulate biological functions. Further, such sequences may be used as part of ribozyme and/or triple helix sequences that are also useful for NHP gene regulation.

Inhibitory antisense or double stranded oligonucleotides
5 can additionally comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-
10 2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine,
15 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine,
20 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide can also comprise at least
25 one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide will comprise at least one modified phosphate backbone
30 selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide
35 is an α -anomeric oligonucleotide. An α -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gautier et al., 1987,

Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-0-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330). Alternatively, double stranded
5 RNA can be used to disrupt the expression and function of a targeted NHP.

Oligonucleotides of the invention can be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from
10 Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides can be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), and methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988,
15 Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

Low stringency conditions are well known to those of skill in the art, and will vary predictably depending on the specific organisms from which the library and the labeled sequences are derived. For guidance regarding such conditions
20 see, for example, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual (and periodic updates thereof), Cold Springs Harbor Press, N.Y.; and Ausubel et al., 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y.

25 Alternatively, suitably labeled NHP nucleotide probes can be used to screen a human genomic library using appropriately stringent conditions or by PCR. The identification and characterization of human genomic clones is helpful for identifying polymorphisms (including, but not limited to,
30 nucleotide repeats, microsatellite alleles, single nucleotide polymorphisms, or coding single nucleotide polymorphisms), determining the genomic structure of a given locus/allele, and designing diagnostic tests. For example, sequences derived from regions adjacent to the intron/exon boundaries of the
35 human gene can be used to design primers for use in amplification assays to detect mutations within the exons,

introns, splice sites (e.g., splice acceptor and/or donor sites), etc., that can be used in diagnostics and pharmacogenomics.

Further, a NHP homolog can be isolated from nucleic acid
5 from an organism of interest by performing PCR using two
degenerate or "wobble" oligonucleotide primer pools designed
on the basis of amino acid sequences within the NHP products
disclosed herein. The template for the reaction may be total
RNA, mRNA, and/or cDNA obtained by reverse transcription of
10 mRNA prepared from human or non-human cell lines or tissue
known or suspected to express an allele of a NHP gene.

The PCR product can be subcloned and sequenced to ensure
that the amplified sequences represent the sequence of the
desired NHP gene. The PCR fragment can then be used to
15 isolate a full length cDNA clone by a variety of methods. For
example, the amplified fragment can be labeled and used to
screen a cDNA library, such as a bacteriophage cDNA library.
Alternatively, the labeled fragment can be used to isolate
genomic clones via the screening of a genomic library.

20 PCR technology can also be used to isolate full length
cDNA sequences. For example, RNA can be isolated, following
standard procedures, from an appropriate cellular or tissue
source (i.e., one known, or suspected, to express a NHP gene,
such as, for example, testis tissue). A reverse transcription
25 (RT) reaction can be performed on the RNA using an
oligonucleotide primer specific for the most 5' end of the
amplified fragment for the priming of first strand synthesis.
The resulting RNA/DNA hybrid may then be "tailed" using a
standard terminal transferase reaction, the hybrid may be
30 digested with RNase H, and second strand synthesis may then be
primed with a complementary primer. Thus, cDNA sequences
upstream of the amplified fragment can be isolated. For a
review of cloning strategies that can be used, see e.g.,
Sambrook et al., 1989, *supra*.

35 A cDNA encoding a mutant NHP gene can be isolated, for
example, by using PCR. In this case, the first cDNA strand
may be synthesized by hybridizing an oligo-dT oligonucleotide
to mRNA isolated from tissue known or suspected to be

expressed in an individual putatively carrying a mutant NHP allele, and by extending the new strand with reverse transcriptase. The second strand of the cDNA is then synthesized using an oligonucleotide that hybridizes
5 specifically to the 5' end of the normal gene. Using these two primers, the product is then amplified via PCR, optionally cloned into a suitable vector, and subjected to DNA sequence analysis through methods well known to those of skill in the art. By comparing the DNA sequence of the mutant NHP allele
10 to that of a corresponding normal NHP allele, the mutation(s) responsible for the loss or alteration of function of the mutant NHP gene product can be ascertained.

Alternatively, a genomic library can be constructed using DNA obtained from an individual suspected of or known to carry
15 a mutant NHP allele (e.g., a person manifesting a NHP-associated phenotype such as, for example, obesity, high blood pressure, connective tissue disorders, infertility, etc.), or a cDNA library can be constructed using RNA from a tissue known, or suspected, to express a mutant NHP allele. A normal
20 NHP gene, or any suitable fragment thereof, can then be labeled and used as a probe to identify the corresponding mutant NHP allele in such libraries. Clones containing mutant NHP gene sequences can then be purified and subjected to sequence analysis according to methods well known to those
25 skilled in the art.

Additionally, an expression library can be constructed utilizing cDNA synthesized from, for example, RNA isolated from a tissue known, or suspected, to express a mutant NHP allele in an individual suspected of or known to carry such a
30 mutant allele. In this manner, gene products made by the putatively mutant tissue can be expressed and screened using standard antibody screening techniques in conjunction with antibodies raised against normal NHP product, as described below. (For screening techniques, see, for example, Harlow,
35 E. and Lane, eds., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Press, Cold Spring Harbor.)

Additionally, screening can be accomplished by screening with labeled NHP fusion proteins, such as, for example, AP-NHP

or NHP-AP fusion proteins. In cases where a NHP mutation results in an expressed gene product with altered function (e.g., as a result of a missense or a frameshift mutation), polyclonal antibodies to NHP are likely to cross-react with a
5 corresponding mutant NHP gene product. Library clones detected via their reaction with such labeled antibodies can be purified and subjected to sequence analysis according to methods well known in the art.

The invention also encompasses (a) DNA vectors that
10 contain any of the foregoing NHP coding sequences and/or their complements (i.e., antisense); (b) DNA expression vectors that contain any of the foregoing NHP coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences (for example, baculo virus
15 as described in U.S. Patent No. 5,869,336 herein incorporated by reference); (c) genetically engineered host cells that contain any of the foregoing NHP coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences in the host cell; and (d)
20 genetically engineered host cells that express an endogenous NHP gene under the control of an exogenously introduced regulatory element (i.e., gene activation). As used herein, regulatory elements include but are not limited to inducible and non-inducible promoters, enhancers, operators and other
25 elements known to those skilled in the art that drive and regulate expression. Such regulatory elements include but are not limited to the cytomegalovirus hCMV immediate early gene, regulatable, viral (particularly retroviral LTR promoters) the early or late promoters of SV40 adenovirus, the lac system,
30 the trp system, the TAC system, the TRC system, the tet system the major operator and promoter regions of phage lambda, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase (PGK), the promoters of acid phosphatase, and the promoters of the yeast α -mating factors.
35 The present invention also encompasses antibodies and anti-idiotypic antibodies (including Fab fragments), antagonists and agonists of a NHP, as well as compounds or nucleotide constructs that inhibit expression of a NHP gene

(transcription factor inhibitors, antisense and ribozyme molecules, or gene or regulatory sequence replacement constructs), or promote the expression of a NHP (e.g., expression constructs in which NHP coding sequences are
5 operatively associated with expression control elements such as promoters, promoter/enhancers, etc.).

The NHPs or NHP peptides, NHP fusion proteins, NHP nucleotide sequences, antibodies, antagonists and agonists can be useful for the detection of mutant NHPs or inappropriately
10 expressed NHPs for the diagnosis of disease. The NHP proteins or peptides, NHP fusion proteins, NHP nucleotide sequences, host cell expression systems, antibodies, antagonists, agonists and genetically engineered cells and animals can be used for screening for drugs (or high throughput screening of
15 combinatorial libraries) effective in the treatment of the symptomatic or phenotypic manifestations of perturbing the normal function of a NHP in the body. The use of engineered host cells and/or animals may offer an advantage in that such systems allow not only for the identification of compounds
20 that bind to the endogenous receptor for a NHP, but can also identify compounds that trigger NHP-mediated activities or pathways.

Finally, the NHP products can be used as therapeutics. For example, soluble derivatives such as NHP peptides/domains
25 corresponding to NHP, NHP fusion protein products (especially NHP-Ig fusion proteins, i.e., fusions of a NHP, or a domain of a NHP, to an IgFc), NHP antibodies and anti-idiotypic antibodies (including Fab fragments), antagonists or agonists (including compounds that modulate or act on downstream
30 targets in a NHP-mediated pathway) can be used to directly treat diseases or disorders. For instance, the administration of an effective amount of a soluble NHP, or a NHP-IgFc fusion protein or an anti-idiotypic antibody (or its Fab) that mimics the NHP could activate or effectively antagonize an endogenous
35 NHP receptor or NHP accessory protein. Nucleotide constructs encoding such NHP products can be used to genetically engineer host cells to express such products *in vivo*; these genetically engineered cells function as "bioreactors" in the body

delivering a continuous supply of a NHP, a NHP peptide, or a NHP fusion protein to the body. Nucleotide constructs encoding functional NHP, mutant NHPs, as well as antisense and ribozyme molecules can also be used in "gene therapy"

5 approaches for the modulation of NHP expression. Thus, the invention also encompasses pharmaceutical formulations and methods for treating biological disorders.

Various aspects of the invention are described in greater detail in the subsections below.

10

5.1 THE NHP SEQUENCES

The cDNA sequences and the corresponding deduced amino acid sequences of the described NHPs are presented in the Sequence Listing. The NHP nucleotides were obtained from
15 human cDNA libraries using probes and/or primers generated from human gene trapped sequence tags. Expression analysis has provided evidence that the described NHP can be expressed in a variety of human cells as well as gene trapped human cells. The NHPs share sequence and structural similarity with
20 uncoupling proteins. Similar proteins have been shown to be associated with regulating metabolism, fat production, and body weight.

5.2 NHPS AND NHP POLYPEPTIDES

25 NHPs, NHP polypeptides, NHP peptide fragments, mutated, truncated, or deleted forms of NHP, and/or NHP fusion proteins can be prepared for a variety of uses, including but not limited to the generation of antibodies, as reagents in diagnostic assays, the identification of other cellular gene
30 products related to a NHP, as reagents in assays for screening for compounds that can be as pharmaceutical reagents useful in the therapeutic treatment of mental, biological, or medical disorders and disease.

The Sequence Listing discloses the amino acid sequences
35 encoded by the described NHP polynucleotides. The NHPs display initiator methionines in DNA sequence contexts consistent with translation initiation sites, and apparently display signal sequences which may indicate that the described

NHP ORFs are secreted proteins or possibly membrane associated.

5 The NHP amino acid sequences of the invention include the amino acid sequences presented in the Sequence Listing as well as analogues and derivatives thereof, as well as any oligopeptide sequence of at least about 10-40, generally about 12-35, or about 16-30 amino acids in length first disclosed in the Sequence Listing. Further, corresponding NHP homologues from other species are encompassed by the invention. In fact, 10 any NHP encoded by the NHP nucleotide sequences described above are within the scope of the invention, as are any novel polynucleotide sequences encoding all or any novel portion of an amino acid sequence presented in the Sequence Listing. The degenerate nature of the genetic code is well known, and, 15 accordingly, each amino acid presented in the Sequence Listing, is generically representative of the well known nucleic acid "triplet" codon, or in many cases codons, that can encode the amino acid. As such, as contemplated herein, the amino acid sequences presented in the Sequence Listing, 20 when taken together with the genetic code (see, for example, Table 4-1 at page 109 of "Molecular Cell Biology", 1986, J. Darnell et al. eds., Scientific American Books, New York, NY, herein incorporated by reference) are generically representative of all the various permutations and 25 combinations of nucleic acid sequences that can encode such amino acid sequences.

The invention also encompasses proteins that are functionally equivalent to the NHPs encoded by the presently described nucleotide sequences as judged by any of a number of 30 criteria, including, but not limited to, the ability to bind or cleave a substrate of a NHP, or the ability to effect an identical or complementary downstream pathway, or a change in cellular metabolism (e.g., proteolytic activity, ion flux or gradient, tyrosine phosphorylation, etc.). Such functionally 35 equivalent NHP proteins include, but are not limited to, additions or substitutions of amino acid residues within the amino acid sequence encoded by the NHP nucleotide sequences described above, but which result in a silent change, thus

producing a functionally equivalent gene product. Amino acid substitutions can be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

A variety of host-expression vector systems can be used to express the NHP nucleotide sequences of the invention. Where, as in the present instance, the NHP products or NHP polypeptides can be produced in soluble or secreted forms (by removing one or more transmembrane domains where applicable), the peptide or polypeptide can be recovered from the culture media. Such expression systems also encompass engineered host cells that express a NHP, or a functional equivalent, *in situ*. Purification or enrichment of NHP from such expression systems can be accomplished using appropriate detergents and lipid micelles and methods well known to those skilled in the art. However, such engineered host cells themselves may be used in situations where it is important not only to retain the structural and functional characteristics of the NHP, but to assess biological activity, e.g., in drug screening assays.

The expression systems that may be used for purposes of the invention include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing NHP nucleotide sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing NHP encoding nucleotide sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing NHP sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV;

tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing NHP nucleotide sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3) harboring recombinant expression
5 constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may
10 be advantageously selected depending upon the use intended for the NHP product being expressed. For example, when a large quantity of such a protein is to be produced for the generation of pharmaceutical compositions of or containing NHP, or for raising antibodies to a NHP, vectors that direct
15 the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which a NHP coding sequence may be ligated individually into the vector in frame
20 with the *lacZ* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors (Pharmacia or American Type Culture Collection) can also be used to express
25 foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The PGEX vectors are
30 designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhidrosis virus (AcNPV) is used as a vector to express
35 foreign genes. The virus grows in *Spodoptera frugiperda* cells. A NHP gene coding sequence can be cloned individually

into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of NHP gene coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed (e.g., see Smith et al., 1983, J. Virol. 46: 584; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the NHP nucleotide sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing a NHP product in infected hosts (e.g., See Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:3655-3659). Specific initiation signals may also be required for efficient translation of inserted NHP nucleotide sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire NHP gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of a NHP coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of

appropriate transcription enhancer elements, transcription terminators, etc. (See Bittner et al., 1987, Methods in Enzymol. 153:516-544).

In addition, a host cell strain may be chosen that
5 modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host
10 cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host
15 cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include, but are not limited to, CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular,
20 human cell lines.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the NHP sequences described above can be engineered. Rather than using expression vectors which
25 contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign
30 DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form
35 foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the NHP product. Such engineered cell lines may be particularly useful in screening and evaluation of

compounds that affect the endogenous activity of the NHP product.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817) genes can be employed in tk⁻, hgp^rt⁻ or ap^rt⁻ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1); and hyg^r, which confers resistance to hygromycin (Santerre, et al., 1984, Gene 30:147).

Alternatively, any fusion protein can be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht, et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972-8976). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni²⁺-nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

5.3 ANTIBODIES TO NHP PRODUCTS

Antibodies that specifically recognize one or more epitopes of a NHP, or epitopes of conserved variants of a NHP, or peptide fragments of a NHP are also encompassed by the invention. Such antibodies include but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')₂ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above.

The antibodies of the invention may be used, for example, in the detection of NHP in a biological sample and may, therefore, be utilized as part of a diagnostic or prognostic technique whereby patients may be tested for abnormal amounts of NHP. Such antibodies may also be utilized in conjunction with, for example, compound screening schemes for the evaluation of the effect of test compounds on expression and/or activity of a NHP gene product. Additionally, such antibodies can be used in conjunction gene therapy to, for example, evaluate the normal and/or engineered NHP-expressing cells prior to their introduction into the patient. Such antibodies may additionally be used as a method for the inhibition of abnormal NHP activity. Thus, such antibodies may, therefore, be utilized as part of treatment methods.

For the production of antibodies, various host animals may be immunized by injection with the NHP, an NHP peptide (e.g., one corresponding to a functional domain of an NHP), truncated NHP polypeptides (NHP in which one or more domains have been deleted), functional equivalents of the NHP or mutated variant of the NHP. Such host animals may include but are not limited to pigs, rabbits, mice, goats, and rats, to name but a few. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human

adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of the immunized animals.

5 Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, can be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique of Kohler and
10 Milstein, (1975, Nature 256:495-497; and U.S. Patent No. 4,376,110), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies And Cancer
15 Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated in vitro or in vivo. Production of high titers of mAbs in vivo makes this the
20 presently preferred method of production.

 In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454) by
25 splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as
30 those having a variable region derived from a murine mAb and a human immunoglobulin constant region.

 Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778; Bird, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad.
35 Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) can be adapted to produce single chain antibodies against

NHP gene products. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

5 Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, such fragments include, but are not limited to: the F(ab')₂ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the
10 disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed (Huse et al., 1989, Science, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

 Antibodies to a NHP can, in turn, be utilized to generate
15 anti-idiotypic antibodies that "mimic" a given NHP, using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, 1993, FASEB J 7(5):437-444; and Nissinoff, 1991, J. Immunol. 147(8):2429-2438). For example
 antibodies which bind to a NHP domain and competitively
20 inhibit the binding of NHP to its cognate receptor can be used to generate anti-idiotypes that "mimic" the NHP and, therefore, bind and activate or neutralize a receptor. Such anti-idiotypic antibodies or Fab fragments of such anti-idiotypes can be used in therapeutic regimens involving a NHP
25 signaling pathway.

 The present invention is not to be limited in scope by the specific embodiments described herein, which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components
30 are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising at least 24 contiguous bases of nucleotide sequence first disclosed in the NHP sequence described in SEQ ID NO: 1.
5
2. An isolated nucleic acid molecule comprising a nucleotide sequence that:
 - (a) encodes the amino acid sequence shown in SEQ ID NO: 2; and
10
 - (b) hybridizes under stringent conditions to the nucleotide sequence of SEQ ID NO: 1 or the complement thereof.
- 15 3. An isolated nucleic acid molecule encoding the amino acid sequence described in SEQ ID NO: 2.
4. An isolated oligopeptide comprising at least about 12 amino acids in a sequence first disclosed in SEQ ID
20 NO:2.
5. An isolated nucleic acid molecule encoding the amino acid sequence described in SEQ ID NO:4.

SEQUENCE LISTING

<110> Turner, C. Alexander Jr.
Mathur, Brian
Zambrowicz, Brian
Sands, Arthur T.

<120> Novel Human Uncoupling Proteins and
Polynucleotides Encoding the Same

<130> LEX-0012-PCT

<160> 4

<170> FastSEQ for Windows Version 3.0

<210> 1

<211> 876

<212> DNA

<213> Homo sapiens

<400> 1

atgtcagccc	tcaactggaa	gccgtttgtg	tacggggggc	tggcctccat	cactgctgag	60
tgccgtacat	ttccaattga	tttaaccaag	acacggctcc	agattcaagg	ccagacgaat	120
gatgcaaaat	ttaaggaaat	tagataccga	ggaatgttgc	acgcattagt	gaggataggg	180
agagaagaag	ggctgaaagc	actctactcg	gggattgccc	ccgcgatgtt	acgccaggca	240
tcctatggca	ccatcaagat	aggcacttac	cagagcttga	agcgactatt	cattgaacgc	300
ccagaagatg	aaactctacc	gataaatgtg	atatgtggaa	ttctgtcttg	agtcatatct	360
tcaaccattg	ctaattccaac	tgatgttttg	aaaattcgga	tgcaagcgca	aagcaacacc	420
attcaaggag	gaatgatagg	caacttcacg	aacatttacc	agcaagaggg	gacaagagga	480
ctgtggaagg	gtgtgtccct	tactgcgcag	agggctgcta	ttgttggttg	tgtggagctg	540
ccggtctatg	acatcaccaa	gaagcatctt	attctctcag	gcctgatggg	agacactgtg	600
tatacccact	tcctctcaag	cttcacctgt	ggctctggcag	gggccctggc	ctcaaaccct	660
gttgatgttg	tgaggacacg	tatgatgaat	cagagagtgc	ttcgagatgg	cagatgttct	720
ggctacacag	gaaccctgga	ttgcttggtt	cagacatgga	agaatgaagg	gttttttgct	780
ctctataaag	gcttttggcc	aaattgggtg	agacttggtc	cttggaatat	cattttcttt	840
gtgacatacg	agcagttgaa	gaaattggat	ttgtga			876

<210> 2

<211> 291

<212> PRT

<213> Homo sapiens

<400> 2

Met	Ser	Ala	Leu	Asn	Trp	Lys	Pro	Phe	Val	Tyr	Gly	Gly	Leu	Ala	Ser
1			5						10				15		
Ile	Thr	Ala	Glu	Cys	Gly	Thr	Phe	Pro	Ile	Asp	Leu	Thr	Lys	Thr	Arg
		20						25				30			
Leu	Gln	Ile	Gln	Gly	Gln	Thr	Asn	Asp	Ala	Lys	Phe	Lys	Glu	Ile	Arg
		35				40						45			
Tyr	Arg	Gly	Met	Leu	His	Ala	Leu	Val	Arg	Ile	Gly	Arg	Glu	Glu	Gly
	50				55					60					
Leu	Lys	Ala	Leu	Tyr	Ser	Gly	Ile	Ala	Pro	Ala	Met	Leu	Arg	Gln	Ala
65				70					75				80		

Ser Tyr Gly Thr Ile Lys Ile Gly Thr Tyr Gln Ser Leu Lys Arg Leu
85 90 95
Phe Ile Glu Arg Pro Glu Asp Glu Thr Leu Pro Ile Asn Val Ile Cys
100 105 110
Gly Ile Leu Ser Gly Val Ile Ser Ser Thr Ile Ala Asn Pro Thr Asp
115 120 125
Val Leu Lys Ile Arg Met Gln Ala Gln Ser Asn Thr Ile Gln Gly Gly
130 135 140
Met Ile Gly Asn Phe Met Asn Ile Tyr Gln Gln Glu Gly Thr Arg Gly
145 150 155 160
Leu Trp Lys Gly Val Ser Leu Thr Ala Gln Arg Ala Ala Ile Val Val
165 170 175
Gly Val Glu Leu Pro Val Tyr Asp Ile Thr Lys Lys His Leu Ile Leu
180 185 190
Ser Gly Leu Met Gly Asp Thr Val Tyr Thr His Phe Leu Ser Ser Phe
195 200 205
Thr Cys Gly Leu Ala Gly Ala Leu Ala Ser Asn Pro Val Asp Val Val
210 215 220
Arg Thr Arg Met Met Asn Gln Arg Val Leu Arg Asp Gly Arg Cys Ser
225 230 235 240
Gly Tyr Thr Gly Thr Leu Asp Cys Leu Leu Gln Thr Trp Lys Asn Glu
245 250 255
Gly Phe Phe Ala Leu Tyr Lys Gly Phe Trp Pro Asn Trp Leu Arg Leu
260 265 270
Gly Pro Trp Asn Ile Ile Phe Phe Val Thr Tyr Glu Gln Leu Lys Lys
275 280 285
Leu Asp Leu
290

<210> 3
<211> 882
<212> DNA
<213> Homo sapiens

<400> 1
atgtcagccc tcaactggaa gccgtttgtg tacggggggc tggcctccat cactgctgag 60
tgcggtacat ttccaattga ttttaaccaag acacggctcc agattcaagg ccagacgaat 120
gatgcaaaat ttaaggaaat tagataccga ggaatgtgc acgcattagt gaggataggc 180
agagaagaag ggctgaaagc actctactcg gggattgccc ccgcgatgtt acgccaggca 240
tcctatggca ccatcaagat aggcacttac cagagcttga agcgactatt cattgaacgc 300
ccagaagatg aaactctacc gataaatgtg atatgtggaa ttctgtctgg agtcatatct 360
tcaaccattg ctaatccaac tgatgttttg aaaattcggg tgcaagcgca aagcaacacc 420
attcaaggag gaatgatagg caacttcatg aacatttacc agcaagaggg gacaagagga 480
ctgtggaagg gtgtgtccct tactgcgcag agggctgcta ttgttgttg tgtggagctg 540
ccggtctatg acatcaccaa gaagcatctt attctctcag gcctgatggg agacactgtg 600
tataccact tcctctcaag cttcacctgt ggtctggcag gggccctggc ctcaaacctt 660
gttgatgttg tgaggacacg tatgatgaat cagagagtgc ttcgagatgg cagatgttct 720
ggctacacag gaaccctgga ttgcttggtta cagcttacag tgctggaaag ttttccacc 780
acaqcaaagc cacaaaagct tatcagcgta gatgccatct cagaagaggc tgataccagg 840
ggatttacat atctcagctg tgatctttct gctccaagct ga 882

<210> 4
<211> 293
<212> PRT
<213> Homo sapiens

<400> 2
Met Ser Ala Leu Asn Trp Lys Pro Phe Val Tyr Gly Gly Leu Ala Ser
1 5 10 15

Ile Thr Ala Glu Cys Gly Thr Phe Pro Ile Asp Leu Thr Lys Thr Arg
 20 25 30
 Leu Gln Ile Gln Gly Gln Thr Asn Asp Ala Lys Phe Lys Glu Ile Arg
 35 40 45
 Tyr Arg Gly Met Leu His Ala Leu Val Arg Ile Gly Arg Glu Glu Gly
 50 55 60
 Leu Lys Ala Leu Tyr Ser Gly Ile Ala Pro Ala Met Leu Arg Gln Ala
 65 70 75 80
 Ser Tyr Gly Thr Ile Lys Ile Gly Thr Tyr Gln Ser Leu Lys Arg Leu
 85 90 95
 Phe Ile Glu Arg Pro Glu Asp Glu Thr Leu Pro Ile Asn Val Ile Cys
 100 105 110
 Gly Ile Leu Ser Gly Val Ile Ser Ser Thr Ile Ala Asn Pro Thr Asp
 115 120 125
 Val Leu Lys Ile Arg Met Gln Ala Gln Ser Asn Thr Ile Gln Gly Gly
 130 135 140
 Met Ile Gly Asn Phe Met Asn Ile Tyr Gln Gln Glu Gly Thr Arg Gly
 145 150 155 160
 Leu Trp Lys Gly Val Ser Leu Thr Ala Gln Arg Ala Ala Ile Val Val
 165 170 175
 Gly Val Glu Leu Pro Val Tyr Asp Ile Thr Lys Lys His Leu Ile Leu
 180 185 190
 Ser Gly Leu Met Gly Asp Thr Val Tyr Thr His Phe Leu Ser Ser Phe
 195 200 205
 Thr Cys Gly Leu Ala Gly Ala Leu Ala Ser Asn Pro Val Asp Val Val
 210 215 220
 Arg Thr Arg Met Met Asn Gln Arg Val Leu Arg Asp Gly Arg Cys Ser
 225 230 235 240
 Gly Tyr Thr Gly Thr Leu Asp Cys Leu Leu Gln Leu Thr Val Leu Glu
 245 250 255
 Ser Phe Ser Thr Thr Ala Lys Pro Gln Lys Leu Ile Ser Val Asp Ala
 260 265 270
 Ile Ser Glu Glu Ala Asp Thr Arg Gly Phe Thr Tyr Leu Ser Cys Asp
 275 280 285
 Leu Ser Ala Pro Ser
 290

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/03425

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C07K14/47 C12N15/12 C12N15/11 C12Q1/68 C07K14/435		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) BIOSIS, EPO-Internal, WPI Data, PAJ, STRAND		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES, 22 September 1998 (1998-09-22), XP002141168 HINXTON, GB AC= A1128486. qc39d07.x1 Soares_pregnant_uterus_NbHPU Homo sapiens cDNA clone IMAGE:1711981 3' similar to SW:UCP2_RAT P56500 MITOCHONDRIAL UNCOUPLING PROTEIN 2 ;, mRNA sequence. EST. abstract <div style="text-align: center; margin-top: 20px;"> --- -/-- </div>	1,2
<div style="display: flex; justify-content: space-between;"> <input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex. </div>		
* Special categories of cited documents : <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search <div style="text-align: center; margin-top: 10px;">27 June 2000</div>		Date of mailing of the international search report <div style="text-align: center; margin-top: 10px;">12/07/2000</div>
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016.		Authorized officer <div style="text-align: center; margin-top: 10px;">Mateo Rosell, A.M.</div>

INTERNATIONAL SEARCH REPORT

Intern al Application No

PCT/US 00/03425

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SANCHIS DANIEL ET AL: "BMCP1, a novel mitochondrial carrier with high expression in the central nervous system of humans and rodents, and respiration uncoupling activity in recombinant yeast." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 51, 18 December 1998 (1998-12-18), pages 34611-34615, XP002141169 ISSN: 0021-9258 the whole document	1-5
A	MAO WEIGUANG ET AL: "UCP4, a novel brain-specific mitochondrial protein that reduces membrane potential in mammalian cells." FEBS LETTERS, vol. 443, no. 3, 29 January 1999 (1999-01-29), pages 326-330, XP002141170 ISSN: 0014-5793 the whole document	1-5
A	DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES, 30 March 1998 (1998-03-30), XP002141171 HINXTON, GB AC =AA881591. vx20e10.r1 Soares 2NbMT Mus musculus cDNA clone IMAGE:1265034 5' similar to SW:UCP2_RAT P56500 MITOCHONDRIAL UNCOUPLING PROTEIN 2 ;, mRNA sequence. EST. abstract	1,2
A	US 5 702 902 A (TARTAGLIA LOUIS ANTHONY) 30 December 1997 (1997-12-30) SEQ.ID.N.56 column 7, line 5 -column 28, line 41; claim 1	1,2
A	WO 98 45313 A (MOORE CANDACE ;ALBRANDT KEITH (US); BEAUMONT KEVIN (US); LARKIN SA) 15 October 1998 (1998-10-15) abstract page 7, line 7 -page 17, line 11; figure 6	1-5
A	WO 98 31396 A (RICQUIER DANIEL ;BOUILLAUD FREDERIC (FR); RECH SCIENT CENTRE DE RE) 23 July 1998 (1998-07-23) abstract page 9, line 20 -page 30, line 10; figure 1A	1-5

-/-

INTERNATIONAL SEARCH REPORT

Intern al Application No

PCT/US 00/03425

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 45438 A (LOWELL BRADFORD B ;FLIER JEFFREY S (US); BETH ISRAEL HOSPITAL (US)) 15 October 1998 (1998-10-15) abstract page 2, line 25 -page 8, line 25; figures 1A-C ---	1-5
P,X	DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES, 17 January 2000 (2000-01-17), XP002141172 HINXTON, GB AC = AW294068. UI-H-BI2-ahb-h-04-0-UI.s1 NCI_CGAP_Sub4 Homo sapiens cDNA clone IMAGE:2726263 3', mRNA sequence. EST. abstract ---	1,2
P,X	DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES, 6 January 2000 (2000-01-06), XP002141173 HINXTON, GB AC = AW274135. xv27b12.x1 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGE:2814335 3' similar to TR:Q9Z2B2 Q9Z2B2 BRAIN MITOCHONDRIAL CARRIER PROTEIN BMCP1. ;, mRNA sequence. EST. abstract -----	1,2

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. al Application No

PCT/US 00/03425

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5702902 A	30-12-1997	US 5861485 A	19-01-1999
		US 5741666 A	21-04-1998
		US 6057109 A	02-05-2000
		US 5853975 A	29-12-1998
		AU 3497295 A	14-03-1996
		WO 9605861 A	29-02-1996
WO 9845313 A	15-10-1998	AU 6585498 A	30-10-1998
WO 9831396 A	23-07-1998	AU 2809797 A	07-08-1998
WO 9845438 A	15-10-1998	AU 7466198 A	30-10-1998